Comparing the Reproductive Success of Yakima River Hatchery and Wild-Origin Spring Chinook

Yakima/Klickitat Fisheries Project Monitoring and Evaluation Report 4 of 7

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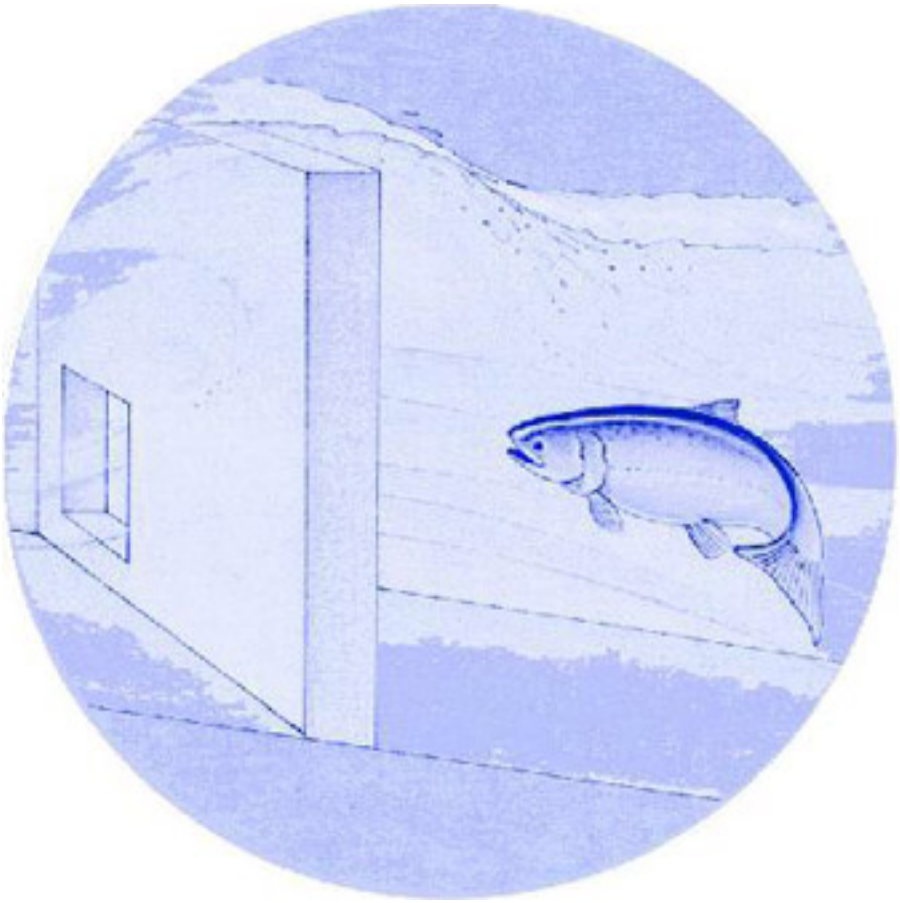

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Comparing The Reproductive Success Of Yakima River Hatchery- And Wild-Origin Spring Chinook

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SUMMARY

In September of 2003, twenty-nine hatchery and twenty-eight wild spring chinook adults were placed into the observation stream located at the Cle Elum Supplementation Research Facility. In, addition 20 precocious males, 7 hatchery and 13 wild, were simultaneously released into the structure. As in previous years, the fish had small amounts of fin material removed prior to being introduced into the stream so that microsatellite DNA based pedigree analyses could be performed on their subsequent progeny. The entire 127 m long by 7.9 m wide stream was made available to this group of fish. Continuous behavioral observations were made while the females prepared nests and spawned. Moreover, standard measurements of adult longevity, spawning participation, water velocity, redd sizes, gravel composition, water temperature and flow were taken. Fry produced from these fish started to emigrate from the stream in early January 2004. They were trapped and sub-sampled for later microsatellite DNA analyses. In mid May of 2004 fry emergence from the channel was complete and residual fish were captured by seine and electro-fishing so that the entire juvenile population could be proportionately sampled.

Audiotape records of the behavior of wild and hatchery adults spawning in the observation stream in 2001 were transcribed into continuous ethograms. Courting, agonistic, and location data were extracted from these chronological records and analyzed to characterize the reproductive behavior of both hatchery and wild fish. In addition, a "gold standard" pedigree analysis was completed on the fry originating from the adults placed into the observation stream in 2001. Behavioral and morphological data collected on hatchery and wild males were linked to the results of the pedigree analysis to ascertain what factors affected their reproductive success (RS) or capacity to produce fry. Individual RS values were calculated for each male placed into the observation stream and the coefficient of variation calculated from these values was greater than 100%. To determine what might be responsible for this degree of variation we examined the relative importance of a variety of physical and behavioral traits. Relative body size, for example, was found not be an important predictor of reproductive success. Instead, the capacity to court females and dominate sexual rivals was directly associated with male RS. However, males that had low dominance scores were also successful at producing offspring. These individuals utilized alternative behavioral strategies to gain close proximity to females and were successful in their attempts to fertilize eggs. Observations made on the color patterns of males showed dominance was closely linked with the possession of an overall black or dark brown color pattern. In addition, we discovered that males that had multiple mates achieved higher RS values than those who spawned with fewer females. The approach we are taking to compare the reproductive competency of hatchery and wild fish is to first determine the factors that are strongly linked to reproductive behavior and then assess whether significant differences occur in the expression of these traits based on the fish origin.

Transcriptions of audiotapes are continuing and a second gold standard pedigree analyses on the fry produced from adults placed into the observation stream in 2002 is nearing completion. Future work will be directed at discovering the factors that affect female RS

values. In the fall of 2004 we will again liberate hatchery and wild fish simultaneously into the entire observation stream to continue our efforts to objectively determine if differences in RS are caused by fish origin.

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INTRODUCTION

Background

The Yakima spring chinook (*Oncorhynchus tshawyscha*) supplementation program is representative of many recovery efforts taking place in the Pacific Northwest that rely on native brood stocks. In this instance, a portion ($\leq$ 50%) of the wild spring chinook returning to the upper Yakima River are taken into the Cle Elum Supplementation Research Facility (CESRF) for breeding and subsequent rearing prior to being released into their natural habitat. The concept of using native broodstock and recycling them through artificial culture until abundance levels increase or become stabilized has been referred to as *supportive breeding* (Laikre and Ryman 1996). It is not without controversy. Behavioral, morphological, and physiological divergences have been observed between wild- and hatchery-adult salmonids (for reviews *see* Fleming and Petersson 2001and Schroder et al. 2003[a]).

It has been suggested that these differences are created by divergent environmental conditions and relaxed or dissimilar selection pressures extant in hatcheries. Such differences may negatively impact hatchery fish when they reside in natural environments (Einum and Fleming 1997; McGinnity et al. 1997; Fleming et al. 2000, Dannewitz et al. 2004). A growing body of literature, for instance, suggests that adult salmon produced by artificial culture are not as reproductively successful as wild fish when they spawn under natural conditions (Hansen et al. 2001; Fleming and Petersson 2001). Dannewitz et al. (2004) point out, however, that many of these investigations compared the reproductive success of non-local hatchery fish with native salmonids or with fish that had experienced multiple generations in a hatchery. Few efforts have assessed reproductive success when both hatchery and wild fish possess a common genetic history (Dannewitz et al. 2004).

Several exceptions have occurred. The reproductive success (RS) of hatchery and wild Atlantic salmon (*Salmon salar*) possessing a common genetic background were compared by Fleming, Lamberg, and Jonsson (1997). They found no differences in the reproductive performance of females. Hatchery males, however, were found to be reproductively inferior to their wild counterparts. How much so depended upon the fish densities and sex ratios they encountered in their breeding arenas. Dannewitz et al. (2004) performed two similar experiments with brown trout (*S. trutta*). In the first one, the RS of seventh generation hatchery fish was compared with wild fish when both were allowed to spawn under natural conditions. No significant differences in RS were found in either males or females. In the second experiment, hatchery environmental effects were controlled by bringing wild fish into a hatchery, breeding them, and selectively marking their offspring. When these F_1 "hatchery fish" returned as mature adults they were allowed to reproduce with seventh-generation hatchery fish. In this instance, males from wild parents were more successful at producing offspring; no differences however, were observed in the females (Dannewitz et al. 2004).

One of the pivotal research objectives of the Yakima Fish Production study has been to compare the reproductive success of fish produced from the Cle Elum hatchery with wild-born conspecifics. In previous reports (Schroder et al 2003[a] and 2003[b]), we have described how such comparisons are being made. Briefly, wild and hatchery-origin spring chinook are liberated into an observation stream located at the CESRF and allowed to reproduce. A suite of comparisons, ranging from body size, spawning ground longevity, spawning participation (as estimated by egg retention and testes weight at death) are made on the fish. Additionally, observers make behavioral records during the spawning period by using audiotapes. Behavioral observations were made during daylight hours and depending upon the number of observers present more than 60 h of recordings were made on each population. Prior to being liberated into the observation stream a small sample of fin material was removed from each fish for microsatellite DNA extraction. Microsatellite DNA was also obtained from sampled juveniles making it possible to estimate the number of offspring produced by each adult fish placed into the observation stream.

The data collected are being used to compare RS values of hatchery and wild fish using a two-step process. First, we are attempting to determine the factors; behavioral, morphological, and physiological, that influence RS in naturally spawning spring chinook. Second, once these factors have been identified comparisons between hatchery and wild fish will be made to assess whether significant differences in these attributes exist. As indicated in Dannewitz et al. (2004) and Schroder et al. (2003[b]) a great deal of variation exists in individual RS values in both male and female salmonids. Consequently, the power to detect differences will be low unless the study populations are large, replication occurs, or major differences exist. So far, we have produced five breeding populations consisting of 10 or more wild and hatchery origin fish of each sex. In four instances, similar densities of fish were allowed to spawn in 46 m long x 7.9 m wide sections of the observation stream. With this type of replication, the power to detect biologically meaningful differences in RS caused by fish origin should be robust.

This past fall the fifth population consisting of hatchery and wild origin spring chinook were allowed to spawn throughout the entire 127m long observation stream. This was done because the first pedigree analyses on the fish placed into the observation stream in 2001 revealed that hatchery females were not as successful at producing offspring as their wild counterparts. We speculated that high instantaneous densities of females in the channel sections produced intra-sexual competition among them for suitable nesting locations. For currently unknown reasons, the hatchery fish did not perform as well under these circumstances. Typically, however, recovery efforts will occur in basins where population densities are low and female competition for space will likely not be intense. To mimic these social conditions, the entire observation stream was made available to 29 hatchery (13 females, 11 males, 5 jacks) and 28 wild (13 females, 14 males, and 1 jack) fish. In addition, twenty precocious males (7 hatchery and 13 wild) were simultaneously released into the stream. In 2004, a similar mixture of hatchery and wild origin fish will be placed into the channel.

Several aspects of this study separate it from other studies designed to compare RS in hatchery and wild salmonids. First, the study fish are from the same population, one that has not previously been subjected to hatchery intervention. Thus, wild spring chinook in the Yakima River, have not been genetically compromised and can serve as representatives of natural salmonids. Second, efforts are being made to characterize the reproductive behavior of the adults to provide causal explanations, if persistent differences in RS between hatchery and wild fish occur. Conversely, such observations may also be used to show similarities in reproductive behavior in the two types of fish. Prior to the advent of DNA-based pedigree assessments behavioral observations were used to infer differences in RS in salmonid fishes. Validation of such appraisals was not readily available. With the advent of this tool, labor-intensive behavioral categorizations are not usually employed. This has occurred because the principle questions being raised are whether differences in RS exist due to treatment origin and not necessarily on why such differences may manifest themselves. In the present study, our goal is to link individual behavior directly to RS in an effort to understand why differences may or may not occur among fish with different life histories.

In early 2004, we began transcribing and analyzing the audiotapes made on fish spawning in the observation stream in 2001. To date, we have completed one set of behavioral observations. It contains over 5,000 observations on fish that were placed into the upper section of the observation stream. In this report, we describe the types of data that were extracted from the audio records. In addition, we present some preliminary findings on the factors that appear to affect RS in males. Specifically we disclose the amount of variation in male RS that was observed in this population. Next, we use the behavioral database to examine the importance of male guarding and courtship activities on their capacity to produce offspring. We then explore the factors that may control acquisition of mates by males by looking at how body size and aggression affect RS. Finally, the capacity to communicate masculine dominance via nuptial color patterns is presented. Comparable evaluations related to female RS will be performed in the future. The results presented here represent our first effort to explore the linkages between the expression of behavior in hatchery and wild males and their RS values. Our notions about how to do this may change as our analyses become more mature. Nonetheless, the current results provide insights into how spawning communities function and indicate what factors appear to drive RS in male spring chinook.

METHODS

Observation Stream

The observation stream located on the grounds of the CESRF is 127 m long by 7.9 m wide and has a "U-shaped" footprint. It is subdivided by eight concrete cross weirs into seven subsections, a curved section or elbow that is 21.m long by 7.9 m wide and six straight sections each measuring 15.2 m long by 7.9 m wide. The stream has banks with 2:1 slopes that are armored with large river rock (10 to 30 cm in diameter) and when it is in operation its wetted width ranges between 4.3 to 5.5 m. The streambed is lined with geotextile to prevent water loss and is filled with 90 cm of double washed stream gravel

that ranges in size from 7.1 mm (0.28 inches) to 100 mm (3.9 inches) in diameter. When the gravel was first placed into the stream in August of 2000 it had a Fredle Index (Lotspeich and Everest 1981) value of 10.6. The stream's water supply is the discharge water from the 18 raceways located at the CESRF. Water from the raceways is pumped into the stream from September through May by using up to four, 25 hp electric pumps and a gate valve regulates flow. Enough water is pumped into the stream to produce velocities that are $\geq$ 0.1 m/sec but less than 1.5 m/sec. In addition, an attempt was made to keep water depths $\geq$ to 0.1 m by using stop logs placed in the cross weirs. These criteria were patterned after the velocities and depths that naturally spawning chinook have been observed to use (Healey 1991; Bjornn and Reiser 1991). Velocity and depth measurements were made at 775 points in the stream in 2001 to determine the proportion of the structure that met these requirements. In addition, Tidbit temperature loggers were placed in the observation stream and they recorded water temperatures once every 2 hrs during the spawning and incubation periods.

To facilitate fish observations, a 2.1 m tall observation wall was installed on both banks of the stream. The wall was built by attaching camouflage netting to three-meter tall fence posts set on 2.4 m centers. Top and bottom rails were attached to the fence posts to help support the camouflage netting. Openings, at eye level, were cut into the netting every 2 meters along its length. Observations made on naturally spawning wild spring chinook in the upper Yakima River showed that both males and females made extensive movements on their spawning grounds. To provide the fish with the opportunity to express this type of behavior we subdivided the observation stream into two equal parts referred to as the upper and lower portions of the stream. Each portion consisted of three of the straight sections that measured 15.2 m long by 7.9 m wide and therefore was 45.6 m long by 7.9 m wide. Every 15.2 by 7.9 m section had a grid system made of 0.6 cm nylon cord that was stretched approximately 30 cm over the surface of the water. The squares in the grid measured 1.5 m wide by 3 m long and each was provided with a unique alphanumeric designation so that fish movements and locations could be recorded. In addition, each of the seven subsections was named. The three uppermost straight sections were called 1.1, 1.2, and 1.3; the curved section was referred to as the elbow while the bottom three sections were identified as sections 2.1, 2.2, and 2.3. A more detailed description of the observation stream can be found in Schroder et al. (2003[a]).

Selection of Hatchery- and Wild-Origin Adults

Spring chinook returning to the upper Yakima River from April through August are randomly selected at the Roza Adult Monitoring Facility and transported to the CESRF where they are held in 30.5 m long by 4.6 m wide by 3 m deep ponds. Beginning in early September the fish are inspected once a week to assess their maturity. Mature fish destined for the observation stream are captured by dip net and anesthetized in a 1:19,000 part solution of MS222 (Bell 1964). Once docile, the fish are weighed to the nearest gram, have fork lengths taken to the nearest mm, and are tagged with numbered 3.8 cm in diameter Petersen Disks. DNA samples are also taken by removing a small amount of fin material from the trailing posterior corner of the dorsal fin. These samples are placed in 100% ethanol and transported to WDFW's genetic lab for microsatellite DNA extraction

and characterization. After being tagged, one or two individuals were placed into an insulated 124 *L* capacity cooler and transported to the observation stream. The entire process from anesthetization to fish liberation took slightly longer than 3 minutes per fish. All the fish placed into a section of the stream were tagged and liberated on the same day; this process usually took three hours or less to complete.

Evaluating Relationships Between Behavioral Traits And Male RS

Assessing Reproductive Success

The reproductive success of each adult fish placed into the observation stream was estimated by performing a pedigree analysis based on microsatellite DNA. This analysis matched the genotypes of prospective parents to those that existed on putative offspring. As indicated above, samples of microsatellite DNA were collected on every adult fish placed into the observation stream. DNA samples were also collected on a randomly selected proportion of the fry that emerged from the observation stream. These were obtained by placing fyke nets with attached live boxes at ends of the upper and lower portions of the stream. The traps were installed in mid-January, several weeks prior to fry emergence to ensure that a representative sample was acquired. The live boxes were checked daily, captured fry were counted, and a sample was taken by randomly removing ten percent of the fry and placing them in pure ethanol. This procedure was continued until fry emergence ceased, at that time, the upper and lower portions of the stream were seined and electro-shocked so that fry rearing in the channel could be counted and sampled. Our goal was to obtain a sample of 1000 fry from each portion of the observation stream. More than this number were collected; therefore the number of fry analyzed from each day's sample was reduced by a consistent percent to produce a 1000 fry sample for each portion of the observation stream. This simple approach meant that the number of fry analyzed for a given day was proportionate to the number of fry captured on that date.

Standard microsatellite DNA methods were employed to determine the genotypes of the parent fish and fry. Template DNA was extracted from whole fry and adult tissues by using chelex resin and microsatellite DNA was selectively amplified by using the polymerase chain reaction. Microsatellite alleles were run on an automated sequencer (ABI 3730) and genotypes were assessed using GENEMAPPER software. CERVUS software was used to assign the sampled fry to the adults placed into the stream (Sewall Young personal communication).

Analysis Of Audio Tapes And Types of Data Collected

Scan and focused behavioral observations (*see* Schroder et al. 2003[a] for more details) were made on the adults while females prepared nests and spawned. During these observations, the location, color pattern, reproductive status, and frequency of a suite of courtship and agonistic behaviors were recorded on the fish being watched. Depending upon the number of observers available, approximately 60 to 90 hrs of taped observations were obtained on each population. The audiotapes were transcribed by hand using

symbols and English. The use of symbols for commonly occurring behavioral activities often allowed the transcriber to keep up with the spoken narrative. While the tapes were being transcribed, a stopwatch was in operation so that the recorder could break the narratives into one-minute segments. At the beginning of each observation period, the date, time, location (channel section and grid name), color patterns, and fish tag numbers were indicated. Scan observations usually lasted for 4 to 5 minutes and on a few occasions focused observations took place. These often lasted for 90 minutes or more and described the interactions occurring around a female while she prepared a nest, spawned, and buried her eggs. Agonistic, courtship, and movement behaviors of the watched fish were described.

The transcribed descriptions were placed into two linked databases. One quantified agonistic behavior while the other recorded courtship activities. In Table 1, an example of the agonistic data format is shown. As can be seen each observation is fish specific. For instance, the top line shows that Male 7 attacked Male 4 in section 1.1 at 11:29 AM. The line directly below this one indicates the same thing except from the perspective of Male 4. The database was sorted by fish number making it possible to sum the number of times an individual attacked or was attacked by neighboring fish.

Table 1. An example of the data form used to quantify agonistic interactions among chinook salmon spawning in the observation stream.

TARGET FISH				ATTACKED FISH								ATTACKED BY FISH								
Date	Sect	Time	Fish #	Fish ID	Total	Fish ID	Total	Fish ID	Total	Fish ID	Total	Fish ID	Total	Fish ID	Total	Fish ID	Total	Fish ID	Total	Color
12-Sep	1.1	1129	M7	M4	1															
12-Sep	1.1	1129	M4									M7	1							
12-Sep	1.1	1129	M28																	
12-Sep	1.1	1130	M14																	Gold
12-Sep	1.1	1130	M27																	
12-Sep	1.1	1131	M23									UM	1							
12-Sep	1.1	1131	F25									M0	1							
12-Sep	1.1	1131	M0	F25	1															Dark
12-Sep	1.1	1132	M0	F17	1															Dark

Six random variables associated with male agonistic or dominance behavior were developed from this data set. A descriptive title and brief explanation on how they were calculated is presented below:

1) *Overall Dominance* equals the number of times a male attacked neighboring fish of either sex divided by the total number of agonistic interactions he experienced. For example, 164 of the 171 agonistic interactions Male 0 experienced were attacks he instigated on other fish. Therefore, his Overall Dominance value equaled 164/171 or 95.9%
2) *Female Attacks* equals the number of observations a male is attacked by one or more females divided by the total number of times he was observed. Table 1, for example, shows that Female 25 attacked Male 0 one time at 11:31 on September

12. Male 0 was observed 200 times and on two occasions was attacked by females. His Female Attack score therefore equaled 2/200 or 1.0%

3) *Attack Frequency* is the mean number of attacks instigated by a male per observation period. Male 0 performed 164 attacks in 200 observation periods therefore his Attack Frequency was calculated to be 164/200 or .82/observation period.
4) *Incidence of Black Color Pattern.* The far right hand column in Table 1 is labeled "color". The general color patterns of both males and females were periodically noted. All of the observations on a given fish were examined and the number that were described as "dark" or "black" for the males was divided by the total number of color observations made. Color assessments that occurred within 10 minutes of one another were counted as one observation. Male 0 had his color pattern noted 26 times, in every instance he was classified as being dark or black giving him a 100% score for this variable.
5) *Proportion of Male Rivals Dominated.* Twenty-five males were in the observed population. Agonistic data were used to determine the number of males out of 24 potential rivals an individual fish could dominate. Attacks were used to assess dominance. Male 0, for example, attacked Male 13 eighteen times and was attacked by this male twice. In this case, Male 0, was judged to be dominant over Male 13. Similar evaluations were made on each of the males he interacted with. If rivals attacked each other an equal number of times they were judged to be equivalent. Using these rules, Male 0 dominated 15 males and thus had a score of 15/24 or 62.5% for this variable.
6) *Dominance Over Individual Opponents.* Males in our populations did not engage in agonistic interactions with all their potential rivals. Consequently, a final dominance indicator was calculated by dividing the number of males an individual dominated by the total number of males he interacted with. Male 0 interacted with 16 males and dominated 15 of them. His Individual Opponent score was thus 15/16 or 93.75%.

Courting behavior in males, exploratory digging, territory establishment, nest building, spawning, and redd guarding in females was quantified by using the data table format shown in Table 2. As in Table 1, the date, section, time, and fish number and sex are entered on each line of the form. The "status" column indicates whether a female is wandering (W), territorial (T) or evicted (E). Males are classified as wandering (W), courting (C), or satellites (S). Wandering females are those fish that have not yet established territories and are moving throughout the observation stream engaged in exploratory digging or using quite water refugias. Conversely, territorial females are engaged in nest construction or guarding their redds while evicted fish are individuals that have lost their territory locations to rival females. Courting or alpha males aggressively defend females and engage in typical courting behaviors. Wandering males are fish that are not associated with females and commonly move throughout the observation stream or rest in quite water zones. Satellites are subdominant males that are closely associated with pairs of fish that are close to spawning. Four columns of data are recorded under Female Data. The first one labeled "grid" is used to record which 1.5 m x 3.1 m grid section is being occupied by a female. Each section has 25 grid sections and

therefore it is possible to track where females dig and whether they use multiple spawning locations, or have been evicted and forced to occupy another location. The "Dig" column is used to record the number of digging activities a female performed while the next column "LTG AFD" (low to gravel, anal fin drag) is used to record whether a female is testing nest depths or nest configuration. The final column for this sex provides an opportunity to generally describe the behavior of the fish. Seven columns are in the male side of the table. The first one, FEM # indicates which female an individual is courting or is associated with as a satellite male. The next five columns are used to quantify the occurrence of common male courting behaviors; quivering (Q), crossing over (CPC), nudging (N), combined nudge and quiver (N&Q), and lying side by side (SBS). The last column is used to give a behavioral sketch of the target fish.

Table 2. An example of the data form used to quantify male courting behavior and territory establishment in female chinook salmon spawning in the observation stream.

TARGET FISH					FEMALE DATA				MALE DATA						
Date	Sect	Time	Fish #	Status	GRID	Digs	LTG AFD	Observations	Fem #	Q's	CPC's	N's	N&Q	SBS	Observations
13-Sep	1.1	920	F8	T	B4			On top of redd mound							
13-Sep	1.1	921	F25	T	B4	1		dug just below F8's location							
13-Sep	1.1	922	F25	T	B4	1		dug in tail end of B-4							
13-Sep	1.1	922	M5	C					F25						courting male for F25
13-Sep	1.1	922	M17	S					F25						satellite male
13-Sep	1.1	923	F25	T	B4	1		dug in tail end of B-4							
13-Sep	1.1	923	F8	T	B4			on mound							
13-Sep	1.1	923	F4	W											
13-Sep	1.1	923	F24	W											

The following three random variables associated with male courting and guarding behavior were generated from these data:

1) *Alpha Male Occurrence.* Alpha males guard females that are preparing nests or provide other cues that they will deposit eggs in the near future. Their close proximity to females, performance of characteristic courting behaviors, and aggressive guarding behavior make them recognizable. To generate an Alpha male score, the number of times a male was observed in this status was divided by the total number of times he was observed
2) *Alpha + Satellite Occurrence.* Some sub-dominant males will position themselves slightly downstream from a courting pair and occupy a "satellite" position. Multiple satellite males may be associated with a pair where they often fight among themselves and the alpha male for proximity to the female. To calculate this variable the total number of times a male was observed as a satellite or alpha male was divided by the number of times he was observed.
3) *Performance of Courting Behaviors.* This variable is determined by dividing the number of observation periods where a male performed one or more courting

displays (e.g. quivering, crossing over) by the total number of times he was observed.

Statistical Analyses Of The Reproductive Success and Behavioral Data

The pedigree analysis assigned parental origins to each sampled fry. A total of 1066 fry were sampled from the 2001 adults that spawned in the upper section of the observation stream. The percentage of the sampled fry fathered by each male was determined and normalized by using the arc sin transformation (Zar 1999). These percentages were used as estimates of each male's reproductive success. Coefficient of Variation values for RS were calculated for the entire male population and for hatchery and wild males separately.

Males that are closely associated with females are expected to achieve relatively high RS values. We examined this hypothesis by using regression techniques. In each of these analyses the dependent variable was male RS while the independent variables were our measures of male courtship; Alpha Male Occurrence, Alpha + Satellite Occurrence, and Performance of Courting Behaviors. Furthermore, results from the pedigree analysis were used to determine how many females produced progeny from each male. These counts were normalized by using the square root transformation (Zar 1999) and regressed against male RS estimates. This allowed us to evaluate whether mate number affected male RS.

Another series of analyses were conducted to explore the importance of relative size and agonistic behavior on male RS. Once again regression analyses were used to appraise the importance of the following independent variables, male body weight and length, and the following agonistic variables: 1) Overall Dominance, 2) Female Attacks, 3) Attack Frequency, 4) Incidence of Black Color Pattern, 5) Proportion of Male Rivals Dominated, and 6) Dominance Over Individual Opponents, on the ability of males to produce offspring.

Two additional tests were performed. First, a number of factors undoubtedly affect the capacity of a male to dominate rivals; one may be the inherent aggressiveness of an individual. Aggressiveness is likely mediated by hormonal levels, social situations, or other factors. Moreover it appears to vary from one individual to the next. As we have seen in the wild males placed into the observation stream in 2000, being relatively large does not necessarily make a male overtly aggressive (Schroder et al. 2003[a]). We examined the effect of aggressiveness on dominance by performing a regression analysis on Overall Dominance (dependent variable) and Attack Frequency (independent variable) to see how important this measure of aggressiveness may be in determining overall dominance in males. Next in previous reports (Schroder et al. 2003[b]), we described how nuptial color patterns in spring chinook can be used to assess their social status. The data we collected on the occurrence of the black color pattern in males was regressed against their overall dominance values to quantitatively assess this apparent relationship.

RESULTS

Evaluating The Importance Of Body Size and Behavior On Male RS

Biological Traits Of The Spring Chinook Placed Into The Observation Stream

On September 12, 2001 twenty-one females (10 hatchery- and 11 wild-origin), twenty-two males (11 hatchery- and 12 wild-origin) and three jacks (2 wild and 1 hatchery) were introduced into the upper section of the observation stream. The ages, size, origin, tag numbers, estimated fecundities and testes weights of the fish placed into the upper portion of the observation stream in 2001 are shown in Table 3.

Table 3. Biological traits of the hatchery- and wild-origin spring chinook placed into the observation stream in 2001 taken from Schroder et al. (2003[b]).

Hatchery- and Wild Origin Females: Upper Portion (Sections 1-1, 1-2, & 1-3)							
Date Introduced To Stream	Type	Age	Tag No.	Weight (Kilos)	Fork Length	Est. Fecundity	Eggs Lost At Tagging
12 Sep 01	HF	4	YY00	3.074	651	3358	0
12 Sep 01	HF	4	YY03	3.916	720	3739	0
12 Sep 01	HF	4	YY04	4.062	729	4084	0
12 Sep 01	HF	4	YY05	5.377	760	6156	8
12 Sep 01	HF	4	YY08	4.403	752	5052	1
12 Sep 01	HF	4	YY13	4.377	754	4492	0
12 Sep 01	HF	4	YY15	4.141	731	3848	0
12 Sep 01	HF	4	YY16	4.435	745	4599	0
12 Sep 01	HF	4	YY17	4.763	754	4714	0
12 Sep 01	HF	4	YY20	3.962	714	4219	0
12 Sep 01	HF	4	YY24	3.546	695	3600	0
12 Sep 01	WF	4	YY01	4.711	724	4335	49
12 Sep 01	WF	4	YY02	6.566	820	6255	21
12 Sep 01	WF	4	YY07	3.892	708	3919	0
12 Sep 01	WF	4	YY09	4.902	768	4266	0
12 Sep 01	WF	4	YY11	2.099	559	1937	0
12 Sep 01	WF	4	YY12	5.086	774	4518	29
12 Sep 01	WF	4	YY14	5.123	763	4451	0
12 Sep 01	WF	4	YY22	3.901	720	3753	0
12 Sep 01	WF	5	YY23	4.962	775	4285	9
12 Sep 01	WF	4	YY25	4.432	742	4094	11

Table 3. Biological traits of the hatchery- and wild-origin spring chinook placed into the observation stream in 2001 continued. . .

Hatchery- and Wild Origin Males: Upper Portion (Sections 1-1, 1-2, & 1-3)						
Date Introduced To Stream	Type	Age	Tag No.	Weight (Kilos)	Fork Length	Estimated Un-spawned Testes Weight (grams)
12 Sep 01	HM	4	WW02	5.207	821	255.5
12 Sep 01	HM	4	WW04	3.401	725	177.8
12 Sep 01	HM	4	WW05	4.952	740	244.5
12 Sep 01	HM	4	WW14	3.844	744	196.9
12 Sep 01	HM	4	WW15	2.776	635	150.9
12 Sep 01	HM	4	WW18	2.143	614	123.7
12 Sep 01	HM	4	WW23	2.229	585	127.4
12 Sep 01	HM	4	WW24	2.910	678	156.7
12 Sep 01	HM	4	WW25	3.215	694	169.8
12 Sep 01	HM	4	WW27	3.207	695	169.5
12 Sep 01	HM	4	WW01	2.259	614	-
12 Sep 01	Hjack	3	WW22	1.452	520	-
12 Sep 01	WM	4	WW00	6.526	842	312.3
12 Sep 01	WM	4	WW03	3.183	696	168.4
12 Sep 01	WM	4	WW07	4.172	756	211.0
12 Sep 01	WM	4	WW08	5.413	844	264.4
12 Sep 01	WM	4	WW09	2.032	630	118.9
12 Sep 01	WM	4	WW11	4.966	813	245.1
12 Sep 01	WM	4	WW12	5.199	805	255.2
12 Sep 01	WM	5	WW13	5.814	870	-
12 Sep 01	WM	4	WW16	2.809	664	152.3
12 Sep 01	WM	4	WW19	4.309	789	216.9
12 Sep 01	WM	4	WW26	7.455	905	352.2
12 Sep 01	WM	4	WW28	4.415	775	221.4
12 Sep 01	Wjack	3	WW17	1.527	520	-
12 Sep 01	Wjack	3	WW31	1.422	525	-

Variation In Male Reproductive Success

Estimates of the reproductive success values of the males placed into the upper portion of the observation stream in 2001 are presented in Table 4. The table shows that variation in male RS is relatively large, for example the coefficient of variation for the entire male population equaled 102.4%. Coefficient of variation values were also determined for each type of male and equaled 88.7% for wild males and 121.3% for hatchery fish.

Table 4. Reproductive success values of the hatchery males (HM), jacks (HJ), wild males (WM), and jacks (WJ) placed into the upper section of the observation stream in 2001.

Male Tag	Male Type	No Of[1] Fry Produced	Estimated Male RS	Arc Sin Of Estimated Male RS
WW01	HM	2	0.0019	7.920
WW04	HM	76	0.0714	15.450
WW05	HM	1	0.0009	1.720
WW14	HM	72	0.0676	15.120
WW15	HM	19	0.0178	7.710
WW18	HM	1	0.0009	1.720
WW23	HM	1	0.0009	1.720
WW24	HM	25	0.0235	8.910
WW25	HM	0	0.0000	0.000
WW27	HM	142	0.1333	21.390
WW22	HJ	32	0.0300	9.970
WW00	WM	181	0.1700	24.350
WW03	WM	3	0.0028	3.030
WW07	WM	1	0.0009	1.720
WW08	WM	13	0.0122	6.290
WW09	WM	1	0.0009	1.720
WW11	WM	1	0.0009	1.720
WW12	WM	247	0.2319	28.780
WW13	WM	54	0.0507	13.050
WW16	WM	3	0.0028	3.030
WW19	WM	14	0.0131	6.550
WW26	WM	37	0.0347	10.780
WW28	WM	1	0.0009	1.720
WW17	WJ	1	0.0009	1.720
WW31	WJ	6	0.0056	4.290

1) Number of progeny produced from the 1066 fry sub-sampled from the observation stream

Relationships Between Male Courting Behavior, Mate Number and Reproductive Success

We examined the relationship between male courting behavior and reproductive success by performing three regression analyses. The three independent variables examined, Alpha Male Occurrence, Alpha + Satellite Occurrence, and Performance of Courting Behaviors, are behavioral proxies for proximity to gravid and active females. In all three

cases, positive relationships between these variables and male RS were observed. As Table 5, shows each of these variables explained about 40% of the variation in male RS. Although we have not yet performed correlations among them, it is likely that all of them are tightly related to one another.

Table 5. Results of the linear regression analyses that evaluated the relationships between the occurrence of male courting activities and RS in hatchery and wild spring chinook spawning in the observation stream.

Regression	n	Slope	Adjusted r^2	F	P value
Alpha male occurrence vs. male RS	25	+0.232	0.439	19.47	0.0002**
Alpha + satellite male occurrence vs. male RS	25	+0.249	0.383	15.92	0.0005**
Performance of courting behaviors vs. male RS	25	+0.353	0.392	16.47	0.005**
Number of Mates vs. male RS	25	+10.577	0.739	68.88	0.000**

r^2 is referred to as the coefficient of determination it indicates the proportion of variation in the dependent variable (Male RS) explained by the independent variable

A single asterisk indicates significance at the alpha 0.05 level, while two asterisks indicate significance at the 0.01 level or greater

Courting and guarding females is calorically expensive for Alpha males. Evolutionary theory predicts that the expenditure of such energy should be directed toward maximizing RS. Several possible alpha male strategies exist, one is to monopolize spawning opportunities with a few females; an alternative tactic would be to attempt to fertilize eggs from as many females as possible. The pedigree analysis allowed us to determine how many different females a male spawned with. A regression analysis was performed and it indicated that there was a strong positive relationship between male RS (the dependent variable) and mate number (Table 5 and Fig 1).

Factors Affecting Male Access To Reproductively Active Females

The above results show that males that can gain close proximity to multiple females will generally achieve high RS values. Relative body size has been identified in previous studies as being a factor that can affect which males have access to females. In general, large males are expected to dominate smaller rivals and thereby gain greater access to females. We examined the effect of male body size by regressing male weight and length against RS values (Table 6). In our population, neither male length nor weight was strongly affiliated with RS. We also used regression analyses to examine how six traits related to agonistic behavior influenced RS in males (Table 6). Five of the tests showed that the examined trait was positively linked to male RS. The strongest association occurred between the random variable Attack Frequency and RS. In this analysis, approximately 50% of the variance in male reproductive success could be explained by

the average number of attacks a male instigated against other males (Fig 2). The only non-significant relationship found was the association between the number of attacks males received from females and their RS values. In aggregate, these tests disclosed that individuals who can dominate most of their potential rivals realize high RS. A number of factors may affect the ability of a male to achieve a high level of dominance. One of the most important appears to be Attack Frequency as this variable explains almost 90% of the variation in male dominance (Fig 3).

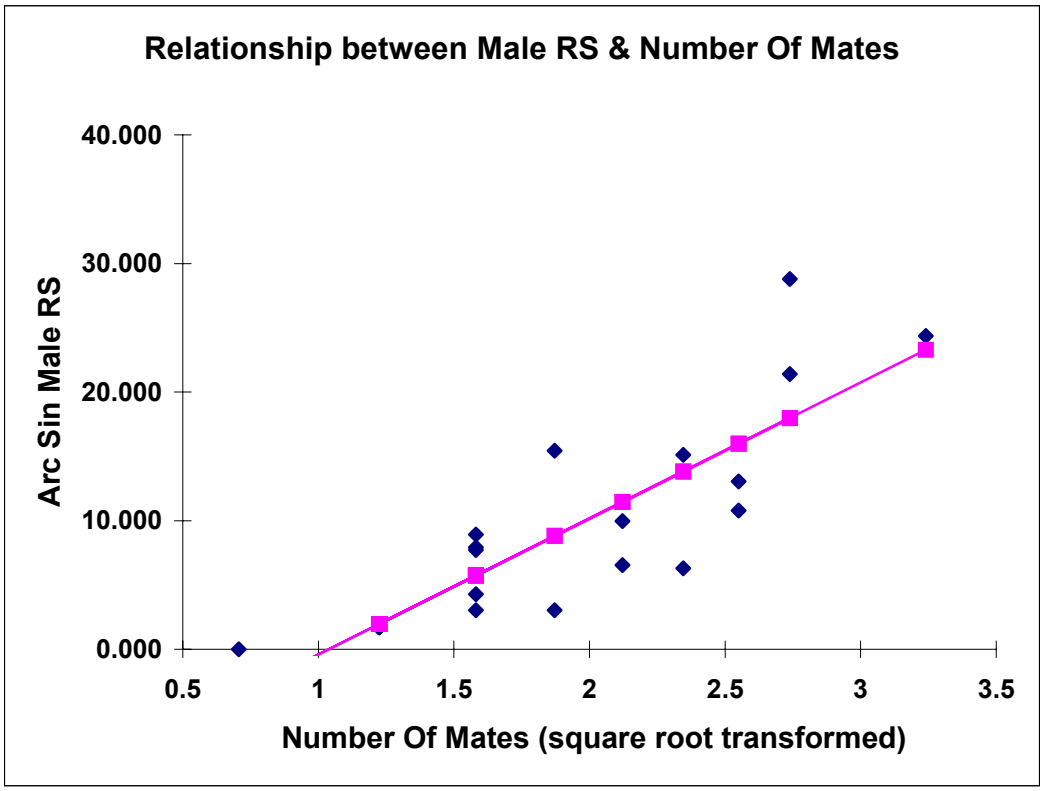

Fig 1. The effect of mate number on male reproductive success in spring chinook spawning in the observation stream..

Table 6. Results of linear regressions between male reproductive success, body size and measures of male and female agonistic behavior in spawning spring chinook salmon spawning in the observation stream.

Regression	n	Slope	r^2	F	P value
Male body weight vs. RS	25	+0.002	0.121	4.301	0.049*
Male length vs. RS	25	+0.026	0.097	3.590	0.071
Overall dominance vs. male RS	25	+0.243	0.383	15.88	0.001**
Incidence of female attacks vs. male RS	25	-0.387	0.100	3.677	0.068
Male attack frequency vs. RS	25	+16.049	0.505	25.456	0.000**
Proportion of total male population dominated vs. male RS	25	+0.322	0.421	18.421	0.000**
Proportion of opponents dominated vs. male RS	25	+0.200	0.392	16.503	0.001**
Percentage of Time in Black color pattern vs. male RS	25	+0.191	0.447	20.392	0.000**

r^2 is referred to as the coefficient of determination it indicates the proportion of variation in the dependent variable (Male RS) explained by the independent variable

A single asterisk indicates significance at the alpha 0.05 level, while two asterisks indicate significance at the 0.01 level or greater

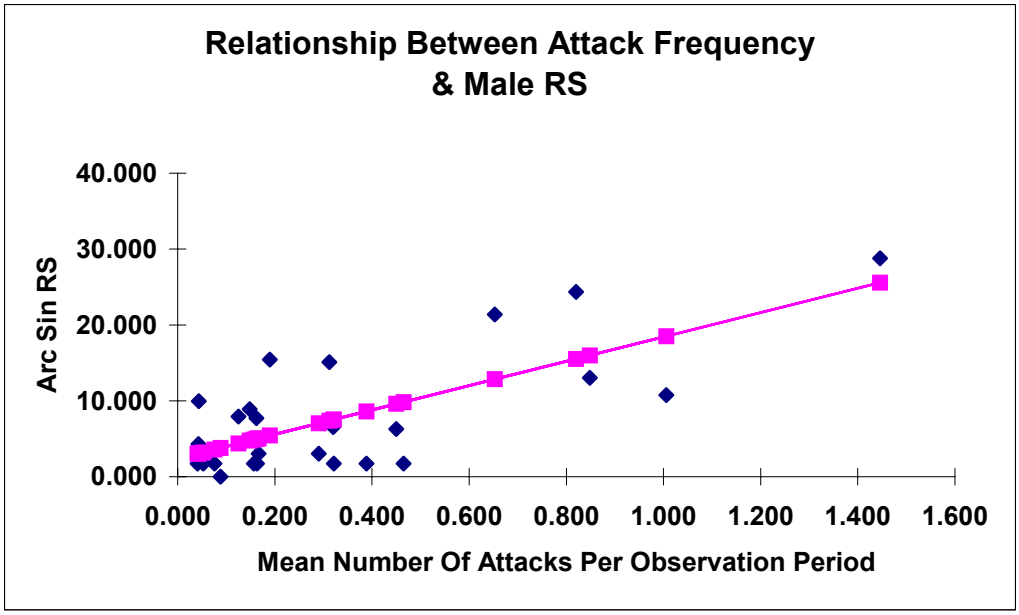

Fig. 2. The relationship between mean number of attacks instigated by males during observation periods and reproductive success.

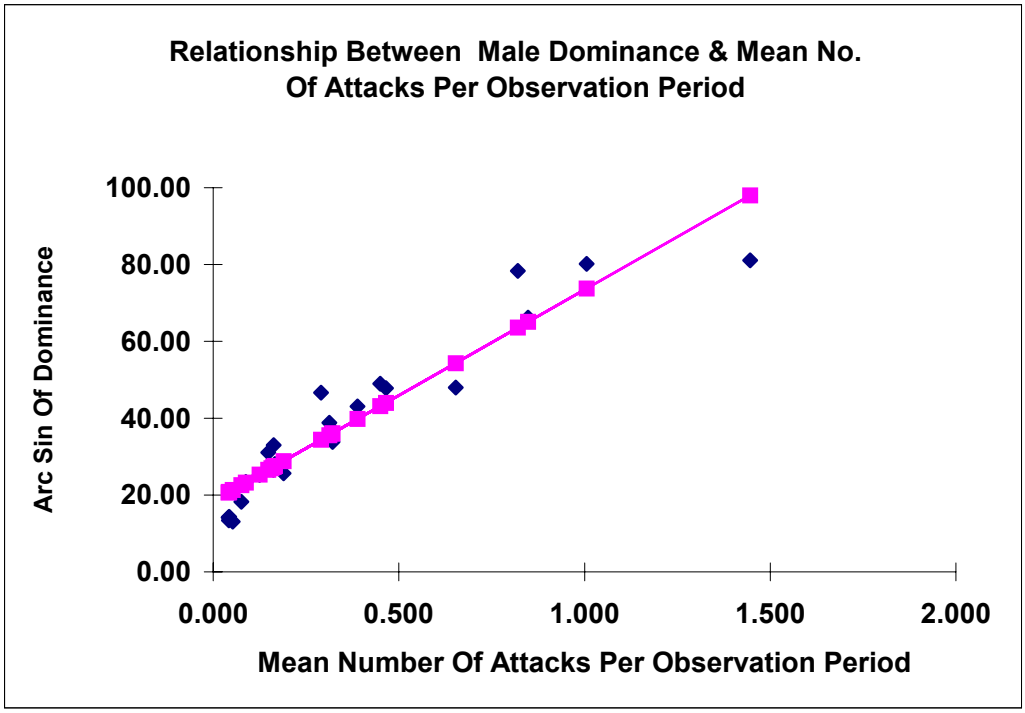

Fig. 3. The influence of attack frequency on male dominance in spawning male spring chinook .

The ability to express dominance in a non-physical way should also be important to males since attacks can be bio-energetically costly. In previous reports (Schroder et al 2003[a] and 2003[b]), we described how nuptial color patterns in spring chinook may reflect

their social status or behavioral state. In Fig 4., the relationship between dominance and possession of the black color pattern in males is illustrated. As the figure indicates, dominant males typically adopt a uniformly dark brown or black color pattern.

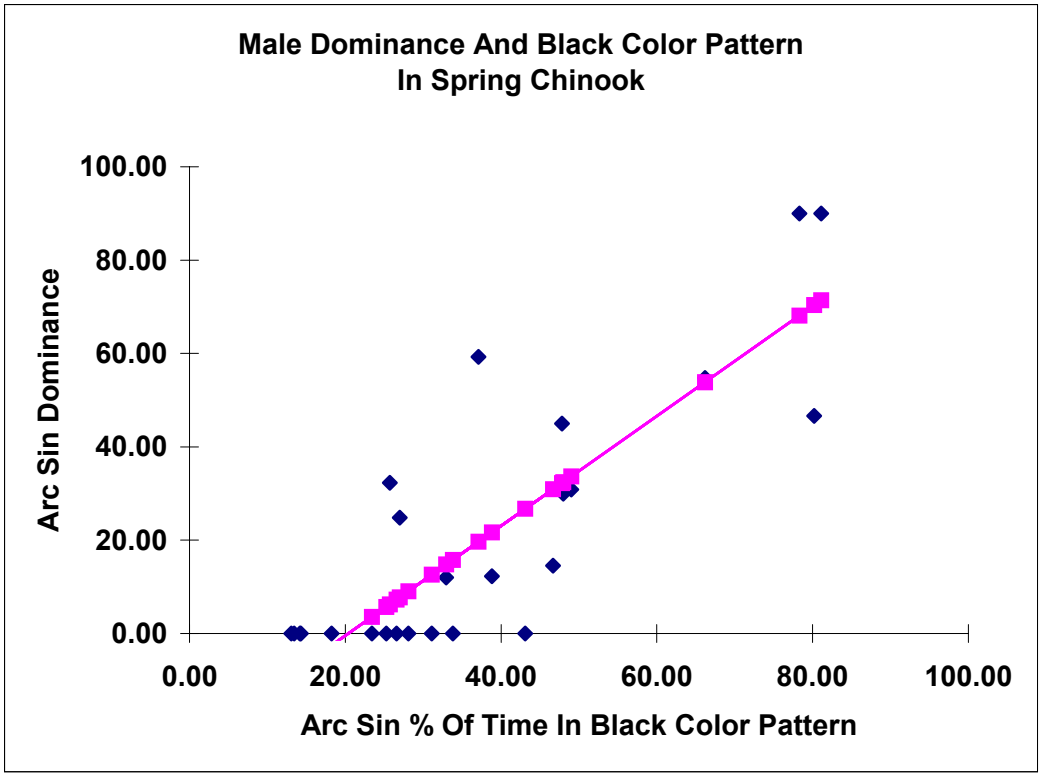

Fig 4. Male dominance and the occurrence of the "black" or uniformly dark brown color pattern in reproducing spring chinook.

DISCUSSION

Our behavioral observations and micro-Satellite DNA based pedigree analyses revealed that a large amount of variation exists in the capacity of both hatchery and wild males to produce offspring. The amount of variation we documented, however was less that what was observed in a pure wild fish population that was examined in the observation stream in 2000 (Schroder et al. 2003[b]). In the 2000 population the coefficient of variation in male RS was approximately 180% as opposed to the slightly more than 100% value observed in 2001. We believe the predominate reason this occurred was that fish spawning in 2000 were reproduced in a 15.2 by 7.9 m wide section of the observation stream while those spawning in 2001 had three times this amount of space. This difference in habitat availability probably provided subdominate males more opportunities to participate in spawning events and to avoid continuous aggressive attacks from males and females. The notion that the extra space reduced deleterious

consequences on males due to aggression is supported by the effect of female aggression on males. In 2000, a regression analysis was performed that examined the relationship between the frequency of female attacks on males and their RS values. In this year, a highly significant, negative relationship was observed that had an r^2 value of .743. A similar analysis was performed on information gathered on the males spawning in 2001. A negative, but non- significant relationship occurred (Table 6). In the 2001 population, the overall incidence of female attacks on males was never higher than 23% for a given male. Conversely, in 2000, four of the ten wild males observed had female attack values that exceeded 39%. The higher incidence of such attacks may have occurred because the fish were confined and had to suffer the consequences of high instantaneous spawner densities.

Table 6 also illustrates that male body size was a relatively unimportant factor in determining RS in the 2001 population. This observation differs from those found by other investigators. For example, Fleming et al. (1996) and Fleming, Lamberg, and Jonsson (1997) discovered that size in Atlantic salmon could account for 23 to 45% of male RS. Fleming and Gross (1994) also report that male size in coho salmon (O. *kisutch*) can significantly affect which individuals have access to spawning opportunities. Moreover, we (Schroder et al. 2003[b]) observed that body size in the pure wild populations placed into the observation stream 2000 was an important factor in determining male RS. Dannewitz et al. (2004) however, found no evidence that male size affected male RS in spawning brown trout. A similar finding for Atlantic salmon was reported by Garant, Dodson, and Bernatchez (2001). Dannewitz et al. (2004) suggest that differences in male densities in the above experiments may help to explain the variation found in the importance of relative size on male RS. Schroder (1981) found that operating sex ratios (number of sexually active males per active female) in chum salmon breeding populations had profound effects on the types of males that had access to females. When OSR values were around one, every male regardless of his relative body size was able to pair with females. When it exceeded 1.5, smaller individuals were excluded from females. At higher OSRs, competition among males became more intense and relative size became even more important. Consequently, localized differences in OSR values in study populations will affect which males have access to females. Additionally, Quinn and Foote (1994) showed that considerable variance in dominance status may occur among salmonid males having similar sizes. Our findings on spring chinook males substantiate their observations. Our data also show the importance that dominance plays in determining male RS. Finally, subdominate males will utilize reproductive strategies that are designed to circumvent intra-sexual competition. Therefore, the capacity to produce offspring is not directly related to being dominant. It is also related to how successful a male has been by using a satellite or sneaker strategy.

This past spring "gold-standard" pedigree assessments were made on the fry produced from the 2001 adults. Similar assessments are currently being finalized for the progeny produced from the fish placed into the observation stream in 2002. We are also continuing to categorize the thousands of individual observation made on the fish while they reproduced. The combination of behavioral records and pedigree assessments are providing us with a unique opportunity to understand the factors that affect RS in spring

chinook salmon. More importantly, the data will allow us to objectively determine if biologically meaningful and persistent differences in the capacity of hatchery and wild fish to produce offspring exist. In this report, we briefly describe some of the factors that affect males. In future reports, these enquiries will be expanded to females.

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